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Case Report

A Case of Olfactory Neuroblastoma Induced in A Rat by *N*-Nitrosobis(2-hydroxypropyl)amine

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Abstract: *N*-nitrosobis(2-hydroxypropyl)amine (BHP) is a well-known carcinogen and induces tumors in various tissues. In the present paper, a case of olfactory neuroblastoma (ONB) induced in a rat by BHP is described. The tumor was observed in one out of 25 male rats that received 2000 ppm of BHP in drinking water from 6 to 18 weeks of age and were sacrificed at 26 weeks of age. Histologically, the tumor arose in the posterior nasal cavity and consisted of small round cells and elongate cells with scant basophilic cytoplasm. The neoplastic cells proliferated with compartmentalization into the lobules by fibrovascular septa. True rosettes, pseudorosettes and an intercellular fibrillar matrix were occasionally observed. Immunohistochemically, the tumor cells were positive for NF120/200 and β III-tubulin. These results indicate that the present tumor is the first case of ONB induced in a rat by BHP treatment. (J Toxicol Pathol 2010; **23**: 111–114)

Key words: olfactory neuroblastoma, *N*-nitrosobis(2-hydroxypropyl)amine, BHP, rat

Olfactory neuroblastoma (ONB) is an extremely rare tumor, that originates from the olfactory epithelium at the posterior nasal cavity. Spontaneous occurrence of ONB has been reported in various animals such as cats[1] and horses[2]; however, no case has been reported in rats. Although chemically-induced ONB is known to occur in rats treated with various nitrosamines including *N*-nitrosonornicotine[3,4], *N*-nitrosopiperazine[3,5], *N*-nitrosomethylallylamine[3], 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone[4] and 1-nitroso-4-methylpiperazine[6], no data concerning ONB induction by *N*-nitrosobis(2-hydroxypropyl)amine (BHP), one of the nitrosamines and a potent tumor initiator in various tissues including the nasal cavity[7,8], have been reported.

The animal was a male Wistar rat purchased from Japan SLC, Inc. (Shizuoka, Japan) at 5 weeks of age and included in a study after a 1-week quarantine period. The present case was one out of 25 male rats treated with BHP in drinking water at 2000 ppm from 6 to 18 weeks of age. Another 25 male rats served as the non-treated control group. The animals were housed individually in stainless steel cages in an environmentally controlled animal room (temperature, 23 ± 3°C; relative humidity, 55 ± 20%; ventilation rate, 10–15 times per hour; and a 12 h:12 h light /dark cycle) and fed a commercial diet (MF-1; Oriental Yeast Co. , Ltd. , Tokyo, Japan) and tap water *ad libitum*. All animals were euthanized under ether anesthesia at 26 weeks of age. The experiment was carried out in accordance with the Guide for Animal Experimentation of Bozo Research Center Inc.

After necropsy, the nose was preserved in 10% phosphate-buffered formalin for histological examination. The nasal tissues were decalcified with a mixture of 20% formic acid and 20% formalin in equal ratio for several days and then trimmed at three levels; level 1 was from the posterior portion of the upper incisor, level 2 was from the portion between the first and second molar teeth and level 3 was from the posterior portion of the ethmoid recess. All tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H.E.). In addition, the sections from the nasal tumor were stained with Masson's trichrome and were also stained immunohistochemically. For immunohistochemistry, the section was stained by the peroxidase-labeled polymer method using an Envision kit (Dako Japan, Kyoto, Japan) for anti-keratin (poly, Dako), anti-desmin (D33, Dako), anti-α-smooth muscle actin (α-SMA, 1A4, Dako), anti-S-100 protein (poly, Dako), anti-neurofilament (NF) 68 (NF68, NR4, Sigma, St. Louis, MO, USA), anti-NF120/200 (NF120/200, RmdO20, Sigma), anti-synaptophysin (poly, Dako), anti-glial fibrillary acidic protein (GFAP, poly, Dako), anti-neuron-specific enolase (NSE, poly, Nichirei, Tokyo, Japan), anti-nestin (Rat-401, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-β III-tubulin (TU-20, Chemicon, Temecula, CA, USA).

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Table 1. Summary of Tumor Incidence in the Rat Nasal Cavity

Group	Control group			BHP-treated group		
Number of rats examined	25			25		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Focal hyperplasia	0*	0	0	1	0	22
Squamous cell papilloma	0	0	0	2	1	3
Adenoma	0	0	0	0	1	1
Neuroblastoma	0	0	0	0	0	1

*Number of rats showing the lesion.

At necropsy, the animals showed no abnormalities in the external macroscopic examination of the nose. However, microscopically, as shown in Table 1, BHP induced various preneoplastic and neoplastic lesions in the nasal cavity, whereas no proliferative lesions were generated in the control group. Microscopically, the present tumor was located in the center of endoturbinate 3 and endoturbinate 4 with invasion of ectoturbinate 2 in the posterior part of the ventral surface in the left nasal cavity at level 3 (Fig. 1). The tumor consisted of relatively homogenous small round cells and partly of elongated cells. The former cells had scant basophilic cytoplasm and round to oval nuclei, which were arranged in lobules and cords compartmentalized by fibrovascular septa. The latter cells had scant basophilic cytoplasm or relatively abundant eosinophilic cytoplasm and oval or elongated nuclei, which frequently formed true rosettes and pseudorosettes (Figs. 2, 3). An intercellular fibrillar matrix was also frequently seen. However, neither a ribbon-like arrangement of the tumor cells, which is one of the characteristic findings of neuroblastoma and primitive neuroectodermal tumor (PNET), nor neurocytoma-like structures were present. Mitotic figure was an occasional finding. Immunohistochemically, the tumor cells were positive for β III-tubulin and NF120/200 (Figs. 4, 5). Both the intercellular fibrillar matrix and tumor cells arranged in solid sheets were particularly strongly positive for β III-tubulin (Fig. 5). On the other hand, the tumor cells were negative for keratin, desmin, α-SMA, S-100 protein, NF68, synaptophysin, GFAP, NSE and nestin.

Among the nasal cavity tumors, the differential diagnosis between ONB and poorly differentiated adenocarcinoma is sometimes difficult due to their morphological similarity. In addition, potent nasal carcinogens including nitroso compounds induce multiple neoplasms that may arise not only from the olfactory epithelium but also from the Bowman's glands[3].

It is generally reported that poorly differentiated adenocarcinomas exhibit a glandular and rosette-like structure composed of anaplastic pleomorphic cells and sometimes exhibit squamous cell differentiation in parts of the tumor. On the other hand, typical findings of ONB are true rosettes, pseudorosettes and plexiform intercellular fibrils. As already mentioned, the present tumor showed some histologic characteristics of ONB. However, the presence of neurogenic components is needed to diagnose ONB. For this purpose, immunohistochemical and electron microscopic examinations as well as histology can provide useful findings for definitive differential diagnosis[9, 10]. The present tumor cells were immunohistochemically positive for β III-tubulin and NF120/200. It is known that β III-tubulin marks developing neurons throughout the central and peripheral nervous systems and is also expressed by both immature and mature olfactory neurons[11]. Previous investigators have reported that some ONB cases showed positive reactions for β III-tubulin and NF[12]. Therefore, the present tumor was diagnosed as ONB based on its histological and immunohistochemical features.

As mentioned above, previous investigators have demonstrated that ONB is induced by various kinds of chemicals such as nitrosamines[3–6], vinyl chloride[13], bis(chloromethyl)ether[14], p-cresidine[15], naphthalene[16], aspartame[17] and type IV phosphodiesterase inhibitor[18]. BHP, a nitrosamine, is known to be a potent tumor initiator in the thyroids, esophagus, pharynx, lungs, liver, pancreas, colon, urinary tract and nasal cavity. In the nasal cavity, it has been reported that squamous cell papilloma, adenocarcinoma and squamous cell carcinoma can be induced by BHP treatment[13,14]. In the present study, animals receiving BHP had various kinds of nasal proliferative lesions including squamous cell papilloma, adenoma, focal hyperplasia and ONB (Table 1). In addition, as already mentioned, spontaneous occurrence of ONB has never been reported in rats. Taken together, it is reasonable to consider that the present tumor is the first reported case of ONB induced in a rat by BHP treatment.

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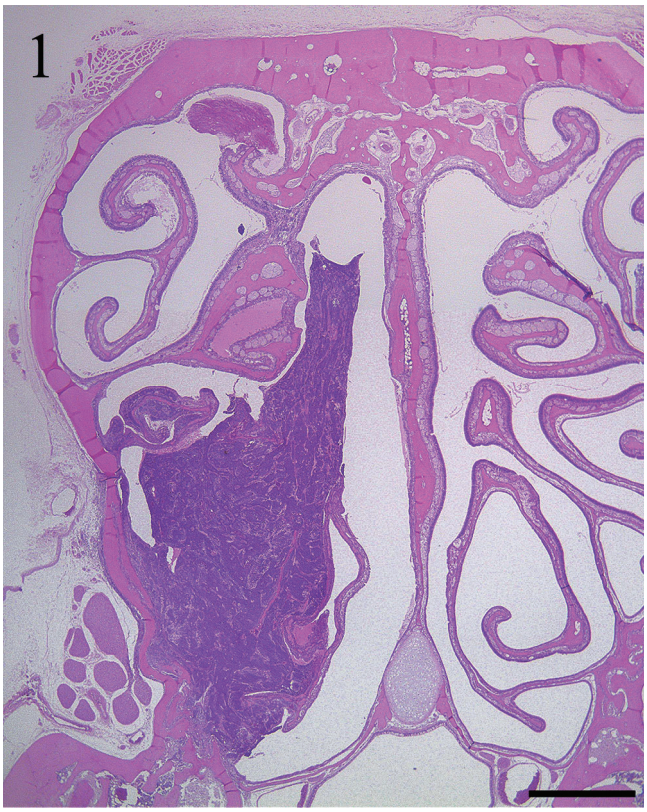

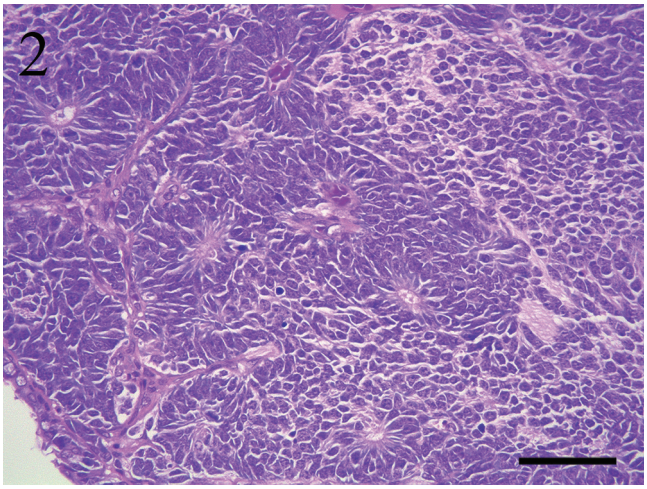

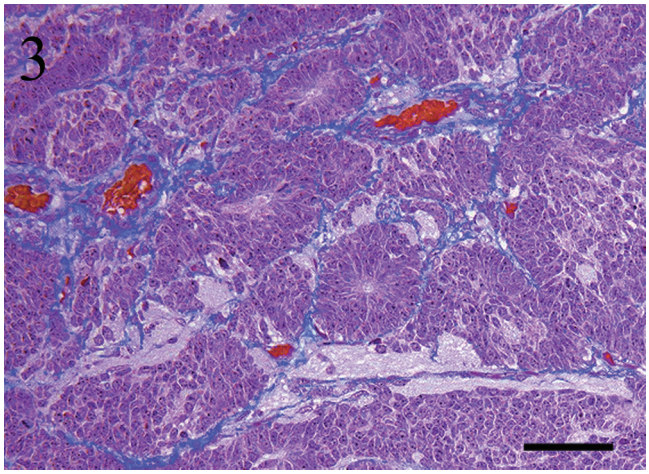

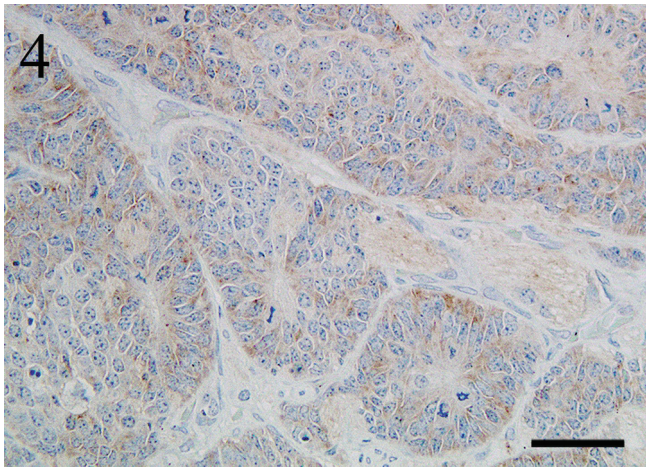

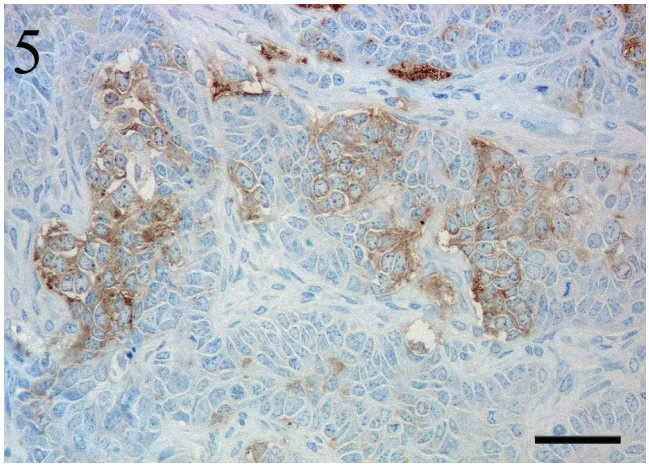

Fig. 1. Location of the tumor in the nasal cavity at level 3. H.E.stain. Bar=1 mm.
Fig. 2. Tumor cells consist of small round cells and elongated cells. True rosettes, pseudorosettes and an intercellular fibrillar matrix can be seen. H.E. stain. Bar=50 μm.
Fig. 3. The tumor is compartmentalized by fibrovascular septa. Masson's trichrome stain. Bar=50 μm.
Fig. 4. Immunohistochemical staining. Tumor cells are positive for NF120/200. Bar=30 μm.
Fig. 5. Immunohistochemical staining. Tumor cells and the intercellular fibrillar matrix are positive for βIII-tubulin. Bar=30 μm.

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